FY98 Investigational Report:

DIAGNOSTIC EVALUATION OF MORIBUND JUVENILE SALMONIDS IN THE TRINITY AND KLAMATH RIVERS (JUNE - SEPTEMBER 1998).



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Summary:

Columnaris and bacterial septicemia were the major disease problems affecting the health and survival of juvenile salmonids in the lower Trinity and Klamath Rivers in 1998. Bacterial infections from Flavobacterium columnare and Aeromonas / Pseudomonas species were isolated most frequently from sick chinook. Water temperature in excess of 20 °Celcius and dissolved oxygen levels below 7 milligram/Liter occurred for extended periods during the June 15th to September 1st study period and are believed to have contributed to the presence of disease. A low estimate of 240,000 natural and hatchery origin chinook perished due to disease prior to their entry into the estuary. We recommend that data on dissolved oxygen and water temperature be collected in the lower Trinity and Klamath Rivers because of their importance to fish health. Additionally, controlled experiments investigating the relationship between Klamath basin pathogens, water quality, and the development of fish disease should be conducted.

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Background

The US Fish and Wildlife Service, California - Nevada Fish Health Center has been an active partner in monitoring health and physiology of outmigrant chinook smolts in the Trinity and Klamath Rivers since 1991. In 1995, a high incidence of severe infection by the enteric parasite Ceratomyxa shasta was observed in moribund chinook smolts captured in the Klamath River (Foott, in preparation). Previous Fish Health Center (1991-1996) studies of Trinity and Klamath river smolts have also detected other important infectious agents including a presporogonic form of an unidentified myxozoan parasite (possible Sphaerospora, Myxidium, or Chloromyxum sp.), a metacercarial stage of the trematode parasite Nanophyetus salmincola, and infection with Renibacterium salmoninarum (the agent which causes bacterial kidney disease). Another abnormality observed in summer outmigrant chinook in the Klamath River is inflammation of the pancreatic tissue and/or associated adipose tissue.

Objective and General Methods The project objectives were to document the magnitude and causes of significant mortality and morbidity in lower Klamath Basin juvenile salmonids (including the lower Trinity River and Klamath Estuary). The study period of June - September 1998 was selected to cover the summer outmigration of juvenile chinook salmon. Site-specific environmental data (temperature, dissolved oxygen, river flow) was also collected or obtained through other sources for possible correlation with disease incidence.

Several species of hatchery and natural fish captured at five sites in the lower Klamath Basin were evaluated for general health. Fish were sampled from rotary screw trap, beach seining, and boat electrofishing operations performed by the US Fish and Wildlife Service, Coastal California Fish and Wildlife Office (CCFWO) and cooperating agencies within the basin (Table 1). Sampling began June 22, 1998 and concluded after 11 weeks on September 1, 1998. Only moribund fish exhibiting clinical signs of disease and pathogen infection were sampled at any particular site. During each site visit, one to 20 lethargic or sick looking fish were examined and sampled for microbiological tests. One to four fish were euthanized at a time, measured for fork length, and examined for external and internal organosomatic characteristics (Goede and Barton 1987, Foott 1990). A numeric "severity" score (0,1,2,3) was assigned to each tissue with 0 equal to normal and 3 representing severe abnormality. The fish's tissue scores were then analyzed for inconsistencies within the sample and compared to scores from what is considered normal (See appendix 1).

If the gill, spleen, or kidney looked abnormal, imprints were made for bacterial diagnosis. Additionally, kidney or spleen tissue were inoculated onto bacterial media (BHIA and/or TYE) for later laboratory identification. Viral samples from the kidney were collected from moribund chinook kidneys and spleen. Posterior kidney was frozen for metacercaria counts using a dissection microscope. Trematode counts were ranked low, medium and high based on 1991-1997 results (Low=1-9, Medium=10-24, and High=> 25 metacercaria in a 100mm Chinook smolt. Category cutoffs are roughly equivalent to 500 and 1000 metacercaria / gram KD, respectively). Bacterial colonies were identified using standard morphological, microscopic, and biochemical identification methods. If Bacterial Kidney Disease was suspect, kidney imprints were analyzed

by the direct flourescent antibody technique (DFAT) for the causative agent *Renibacterium* salmoninarum. For histology, the intestinal tract, kidney, gill, and liver were removed and fixed in Davidson's fixative (Humanson 1979), processed for 5μ m paraffin sections, and stained with hemotoxylin and eosin. Tissue abnormalities and parasite infections were evaluated by light microscopy. Rotary screw trap and estuary crews also recorded the number of fish with external signs of disease.

Table 1. Sample site, operators and global positioning system coordinates.

Sample site	Operator	Global positioning system coordinates
Willow Creek Trap-mainstem Trinity River (rkm = 34)	US Fish and Wildlife Service, Coastal California Fish and Wildlife Office	N40:59:12.3 W123:38:5.77, Elevation ~320 ft.
Weitchpec Trinity River trap (rkm 0)	Yurok Tribal Fisheries, Klamath, CA	N41:11:04.77, W123:42:22.39, Elev172 ft (+-39ft)
Omagar Creek Trap-mainstem Klamath River (rkm 16.9)	Yurok Tribal Fisheries, Klamath, CA	N41:29:19.12, W123:57:49.17, Elevation 6ft (+-45)
Big Bar Trap-mainstem Klamath River (rkm = 81)	US Fish and Wildlife Service and Karuk Tribal Fisheries, Somes Bar	N41:15:2.87 W123:38:21.87 Elevation ~400ft.
Klamath Estuary (rkm 0)	California Department of Fish and Game, Arcata, CA	N41:32:20.38, W124:04:31.5, Elev20ft (+-12ft)

Saltwater Challenge - A 72 hour saltwater challenge was performed on August 27, 1998 to see if a relationship existed between the osmoregulatory ability of challenged chinook and the intensity of a myxosporidian kidney infection. Myxosporidian kidney infection was quite common in Klamath Estuary chinook in 1997 and was associated with glomerulonephritis. Fifteen juvenile chinook were captured alive in the upper estuary by boat electroshocking, transported to the CCFWO in an aerated bucket of cooled (17°C) estuary water, and placed in a 106 liter aquarium containing aerated, 27 ppt saltwater (INSTANT OCEAN TM) at 17°C. After 72 hours, surviving fish were measured, weighed, bled for a plasma sodium sample and the kidney fixed for histological examination.

To estimate the number of mortalities in the outmigrant chinook population associated with disease, the mortality rates of chinook at the Trinity and Klamath river rotary screw traps were used to estimate the hatchery and natural juvenile chinook population mortality. Trap mortality can be related to a number of factors such as handling stress, high fish density, and disease. To generate a hatchery origin juvenile chinook mortality estimate, the percent mortality in the rotary screw trap catch was multiplied by the total hatchery release figure to generate a total population-wide mortality estimate. For the natural origin juvenile chinook mortality estimate, the proportion of adipose fin clipped chinook to unmarked chinook in the hatchery release group was compared to the proportion recovered in the rotary screw trap. The difference in proportions is the natural

component (less approx. 3000 marked Trinity River natural chinook). Using this proportion, the natural origin percentage of the catch can be predicted. This percentage multiplied by the hatchery release number produces a natural chinook population figure which can be multiplied by the trap mortality rates. Four assumptions are detailed which may affect population mortality estimates. They are:

- 1) the primary pathogens isolated in this study are the same pathogens responsible for the majority of the mortality at the traps.
- 2) Klamath and Trinity river rotary screw trap mortality rates are equal to the mortality rate occurring in the migrating chinook population. In other words, there are no differences in the occurrence of dead chinook in the trap's catch and the occurrence of dead chinook in the population.
- 3) the occurrence of adipose fin clipped chinook in the trap is equal to the rate of occurrence in the population.
- 4) adipose fin clipped hatchery and natural juvenile chinook have equal mortality rates and there is no behavior differences resulting from their marking which affects their survival.

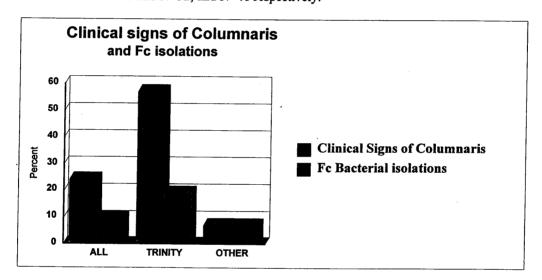
Results

Two hundred seven fish were examined, of which 193 were chinook salmon (Oncorhynchus tshawytscha). Other fish species sampled include sockeye salmon (kokanee?) (Oncorhynchus nerka), steelhead/rainbow (Oncorhynchus mykiss) and cutthroat (Oncorhynchus clarki) trout, ammocoete larvae of Pacific lamprey (Lampetra tridentata), Klamath small scale sucker (Catostomas rimiculus), and speckled dace (Rhynichthys osculus). Analysis of data regarding species other than chinook is limited since they were far less prominant in the data compared to chinook. Additionally, since only ten fish were sampled at the Trinity River Weitchpec screw trap and because it is relatively close to the Willow Creek mainstem Trinity River trap, the findings from this trap are usually lumped together with the Trinity River Willow Creek rotary screw trap.

Bacteria Results - A *Flavobacterium* species (presumably *Flavobacterium columnare*) was associated with external lesions resembling Columnaris disease. This disease was observed in a large portion of the moribund fish examined in this study. While only isolated in culture from 10% (8 out of 78) of all bacterial samples taken, its clinical signs of necrotic gill and yellowish lower jaw were observed in 24% (34 out of 139) of the fish examined in the study. Symptoms of columnaris were noted in 57% (25 out of 44) of samples from the Trinity River Willow Creek trap. Furthermore, bacterial samples from this location produced *F. columnare* isolates in 6 out of 32 samples (18%) (see chart 1). Average daily water temperatures on the lower Trinity and Klamath Rivers ranged between 12.6 and 25.4 degrees Celcius during the study period (measured every two hours by Onset Dataloggers at Trinity River (rkm 34) and Klamath River (rkm 81)). The most frequently occurring daily average temperatures during the study period were 23.5 and 21.0 for the Klamath and Trinity Rivers respectively.

Columnaris disease is generally lethal and tends to attack fish at relatively high water temperatures. Holt et al. (1975) demonstrated that *F. columnare* infection at temperatures above 17.8 °C resulted in over 50% mortality in juvenile chinook. At 23.3 °C, the challenged chinook incurred a 92 % mortality. Field studies in the Columbia River also support the relationship of elevated water temperature and columnaris. Pacha and Ordal (1970) reported a dramatic increase in the incidence of columnaris in adult sockeye salmon during times of high water temperatures. The potential for *F. columnare* to inflict disease in the lower Klamath Basin is high given that these reported water temperatures are a common feature of the basin in late spring and summer.

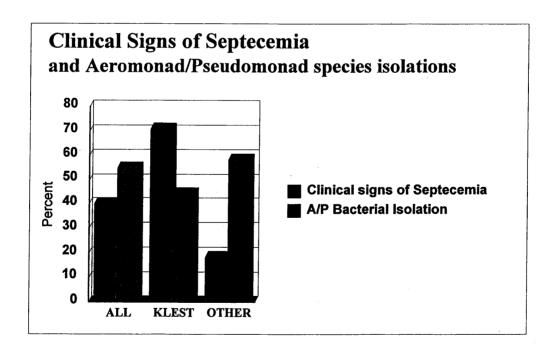
Chart 1. Percent of moribund fish examined with clinical signs of columnaris disease and of bacterial sample isolation of *Flavobacterium columnare* (Fc) from all sites (ALL), Trinity River rotary screw traps (TRINITY), or sites other than the Trinity River s (OTHER). The number of fish examined for clinical signs of columnaris from ALL (n=139), TRINITY (n=44), and OTHER sites (n=95). The number of bacterial samples taken from fish at ALL, TRINITY, and OTHER sites are N=78 and N=32, and N=46 respectively.



Systemic bacterial infections (septicemia), characterized by subcutaneous hemorrhaging at the fin bases, abdomen, and mouth were also common disease symptoms seen in sick fish. Clinical signs of septicemia were seen in 40% (62 out 155) of the all sick fish examined (chart 2). This lesion was quite prevalent in the Klamath Estuary (71%, 37 out of 52 fish examined). Fifty-five percent (43 out of 78) of the bacteria cultured from sick fish at all sites were either *Aeromonas* and *Pseudomonas* species (A/P) or *Vibrio* type bacteria. Since *vibrio* type bacteria are biochemically similar to A/P and cause similar symptoms, a subset of theses cultures (6 out of 78) was characterized by the California-Nevada Fish Health Center using antibiotic sensitivity tests. No samples were identified as *vibrio* spp. Furthermore, *vibrio* species of bacteria do not grow well on aqueous bacterial media without 1.5-2.0 % sodium chloride (Warren, 1991). Since, no

sodium chloride was used in bacterial media for this study, vibrio is assumed to be absent from any of our A/P isolates. Nevertheless, the possibility remains that A/P isolates was misidentified. In the Klamath estuary sample group, A/P isolations occurred in 44% (8 out of 18) of the bacterial samples. At sites other than the Klamath estuary, it was common (58%) to isolate A/P bacteria from kidney samples, however, only 18% of the sampled fish from these other sites showed clinical signs of septicemia. This data indicates most of the fish examined had sub-clinical A/P infections except in the Klamath Estuary. Aeromonas and Pseudomonas pathogens are opportunistic and ubiquitous in soil and water. The mortality rate due to A/P septicemia infections is unknown, but it is likely that survival was affected by this infection. Aeromonas hydrophila, Aeromonas punctata, and Pseudomonas fluorescens are the species assumed to be responsible for these types of infection.

Chart 2. Percent of examined sick fish with clinical signs of septicemia and bacterial samples resulting in isolation of *Aeromonas/Pseudomonas* species. Number of fish with clinical signs from all sites (ALL, n= 155), Klamath estuary (KLEST, n=52), all other non-estuary sites (OTHER, n=103). Bacterial samples from all sites, Klamath estuary, and other non-estuary sites were n=78, n=18, and n=60, respectively.



Renibacterium salmoninarum The bacteria causing bacterial kidney disease was visualized by direct fluorescent antibody technique (DFAT) from only four out of 14 chinook kidney samples. No other isolations of this pathogen were made and like recent years, it was not considered a problem in the Klamath Basin in 1998.

Parasites Results- A metacercarial stage of a trematode parasite (presumptive identification *Nanophyetus salmincola*) was detected in squashes of kidney tissue and histological samples of gill and kidney taken from all sample groups. Trematode infections of gill or kidney were judged not to be a significant health problem (few "high"intensity infections) during 1998 (see chart 3) when compared to 1991-1994 collections. Like previous years, a higher percentage of Trinity River chinook (and one steelhead) had "medium" and "high" kidney metacercaria infections compared to Klamath River chinook. Histological analysis revealed metacercaria in 47% of all the sampled gills and 58% of the gills from the Klamath River at the Big Bar Rotary screw trap (see chart 4). For some reason, a higher percentage of gill metacercaria infections occurred in Klamath fish rather than Trinity origin chinook. In contrast, a higher number of kidney metacercaria infections were detected in the Trinity chinook.

Average daily flow in the Trinity and Klamath Rivers in 1998 was the highest or second highest since 1988 (see appendix 2). We theorized that slower flows would result in higher numbers of infected intermediate hosts (*Juga* sp. snail). A reduction in scouring flow would have a positive impact on snail survival, distribution, and age composition. Older snails have a higher incidence of infection and more parasites than younger snails. Any ecological condition which favors older or more snails will likely result in higher infection rates in smolts. Since flows were above average during 1998, snail density and infections rates were probably not significant to smolt survival. For more information on the metacercarial infections (see appendix 3).

Chart 3. Percentage of smolts with kidney metacercarial infection and relative intensity of infection from all (ALL) samples, all Trinity River (TR) samples, Klamath River samples at Big Bar (KLBBT) and Omagar Creek (KLOC) trap sites, and the Klamath Estuary (KLEST). The infection intensity is based on the number of metacercaria per kidney that fall into corresponding categories (Low = 1-9, Medium = 10-24, High => 25).

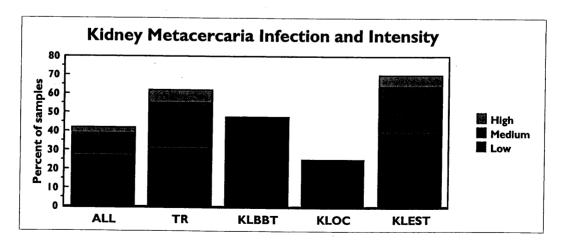
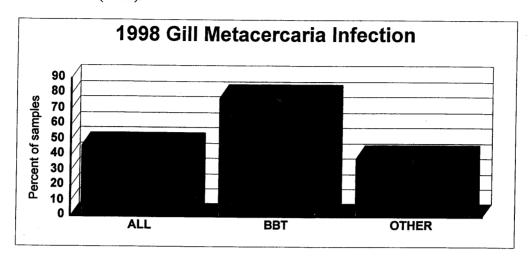


Chart 4. Percentage of smolts with gill metacercarial infection (histological samples) by site. "ALL" represents all gill samples (N=66) from all sites, "BBT" are samples from the Klamath River at the Big Bar Rotary screw trap (N=9), and "OTHER" are samples from the sites other than the BBT site (N=57).

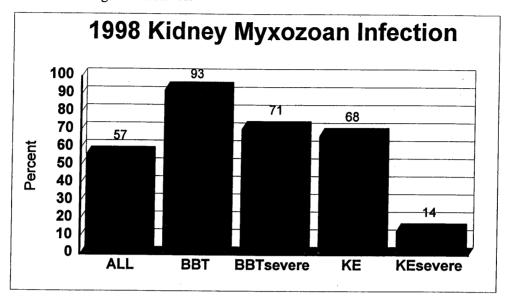


Unidentified Myxozoan Parasite - The previously seen, but unclassified myxozoan parasite was noted by histology again in 1998. Fifty-eight percent of all sampled fish had pre-sporogonic stages of a myxozoan parasite in the glomerulus and tubules of the kidney. Sample sites with the most significant infections were at the Klamath River Big Bar site in July and the Klamath Estuary in August. Ninety-four percent of samples from the Klamath River, Big Bar site had the infection, and 76% were judged "severe" based on their association with tissue damage. Seventy percent of the Klamath Estuary sample kidneys had this infection, but only 23% were considered severe (see chart 5). In contrast, only seven percent of Trinity River captured chinook (from the Weitchepek and Willow Creek rotary screw-trap) had evidence of myxozoan infection. The effect of this parasitic infection and the associated glomerulonephrititis on smolt survival is unclear. Mild infections do not appear to impair osmoregulation in saltwater (Appendix 4).

The enteric parasite *Ceratomyxa shasta*,, which in 1995 caused severe infections and mortality in Klamath River chinook, was not often detected in 1998. Sampling bias may have reduced the detection ability in 1998. The Klamath River rotary screw traps were either poorly located or non-functioning during most of August, 1998. Therefore, any *C. shasta* associated health problems may have gone unnoticed. External parasites such as copepods (e.g., *Salmincola*) and glochida (larval mussel stage infection of gill) were also observed in the fish. Copepods were seen on 18% (8 out of 44) of observed gills in the Trinity River chinook and may have predisposed fish to columnaris.

Viral samples did not produced evidence of virus in any samples in this study (no cytopathic effect after 14 days). Sample dates, sites, and results follow: 1) 8/11/98, Trinity River, Willow Creek Trap, 0/10 chinook, 2) 8/24/98 Klamath Estuary, 0/2 steelhead, and 3) 8/31/98, Klamath Estuary, 0/5 chinook.

Chart 5. Myxozoan parasite infection of kidney from all sites (ALL) (N=63), Klamath River at Big Bar site (BBT) (N=14), Klamath Estuary (KE) (N=28), and "severe" infections at those sites when tissue damage was observed.



Water Quality - The Klamath River at river kilometer 81 had dissolved oxygen levels below the acute threshold of 7 mg/L set by the Environmental Protection Agency (1986) for a total of 175 hours over several days between 17 August to 31 August, 1998 (see chart 6). Additionally, dissolved oxygen conditions on both the lower Klamath and Trinity Rivers were below the 8 mg/L standard set by the State of California, North Coast Water Quality Control Board (1993) for most of the month of August. No dissolved oxygen data was collected in September, but similar conditions probably persisted into the first week when air temperatures were above 32° Celsius in the Klamath Basin. Dissolved oxygen was expected to be at its worst for fish during the late night or early morning hours of the study period when biological respiration is highest. Unfortunately, additional round-the-clock dissolved oxygen monitoring was not possible on the Klamath River due to a lack of monitoring equipment.

When adverse environmental conditions exist, like high water temperatures, bacteria are more likely to cause serious fish disease. For example, columnaris is more likely to cause problems above 12.7 °C (Holt et al. 1975) and infections can be explosive above 18 °C (Warren 1991). Temperatures during this study were above 18 °C on both the Trinity and Klamath Rivers from July 1, 1998 to September 1st (see chart 7). Klamath River water temperatures were measured in excess of the 20 °C elcius Environmental Protection Agency standard (1986) for 68% of the time between June 15th and Septmeber 1st, 1998 (53.6 days). Since the dissolved oxygen and temperature conditions measured by the Hydrolab ® and the Onset ® dataloggers were probably similar throughout the lower Klamath River, the performance of fish (swimming capacity, energy reserves, disease resistance) was most likely compromised. Despite the relatively good water flow regime during 1998 compared to the previous 11 years (USGS flow gauging data 1989-1998, see appendix 2), elevated water temperature and low dissolved oxygen are believed to have

a role in 1998 fish health problems.

Chart 6. Dissolved Oxygen on the Klamath River at Big Bar Trap (rkm 81) from August 17 through September 1, 1998. (Recorded every 30 minutes).

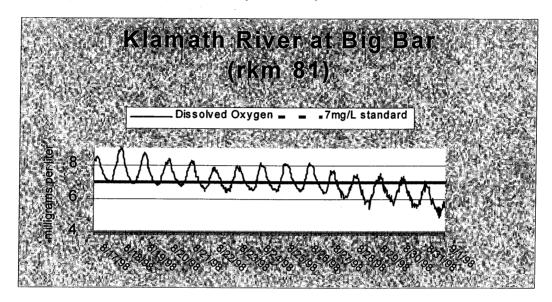
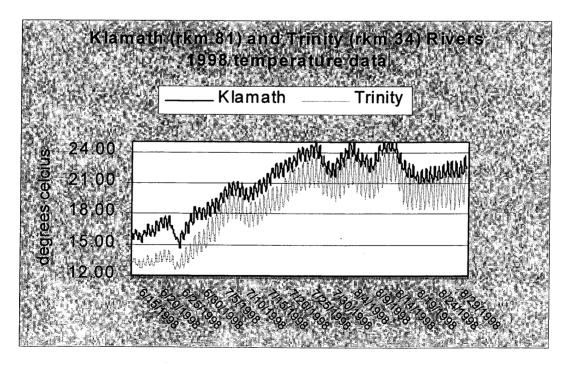


Chart 7. Water Temperatures recorded every two hours at Klamath River at Big Bar (rkm 81) and Trinity River at Willow Creek (rkm34) from June 15 through September 1, 1998.



Discussion and Mortality Estimate

Flavobacterium columnare and Aeromonas / Pseudomonas bacteria were responsible for the columnaris and septicemia observed in moribund fish from the lower Klamath Basin during 1998. Columnaris seemed to affect Trinity River chinook more than Klamath River chinook while septicemia was more prevalent in the Klamath Estuary. The elevated temperature and low dissolved oxygen conditions found in this study may have contributed to pathogen infections, disease, and mortality. Parasite infections were not judged to be a significant source of disease in 1998.

Both hatchery and natural fish are infected by a wide array of pathogens throughout their life cycles which may or may not produce disease. In general, fish disease results when a virulent pathogen invades a susceptible host that has been weakened by stressful environmental conditions and is unable to mount a physiological defense. Pathogens like *Flavobacterium columnare* and *Aeromonas / Pseudomonas* species, are opportunistic and always present in the environment. Aeromonads are common microbes in the intestinal tract of salmonids (Ringo et al. 1995). Their abundance is influenced by changes in environmental conditions. Fish disease from these and other pathogens act to weaken the fish and impair its performance or cause direct mortality. Mesa et al. (1998) report an increased predation rate experienced by chinook with BKD. We would expect a similar effect on Klamath Basin chinook undergoing a severe pathogen infection.

Mortality rates between June 15th and September 1st, 1998 were 1.4 and 2.1 % at the respective Fish and Wildlife Service operated Trinity and Klamath river rotary screw traps. Approximately 4.3 million Trinity hatchery and 5.1 million Klamath hatchery chinook were released during the June 15 to September 1 time period (California Department of Fish and Game memorandum, 1998). One point four percent of the Trinity Hatchery chinook population is approximately 61,000 (see table 2). Two point one percent of the Klamath Hatchery chinook population is approximately 107,000.

An estimated 51% of Trinity River Trap caught chinook and 38% of Klamath River Trap caught chinook were of natural origin during the period 6-15-98 to 9-1-98. This translates to an estimated 2,224,412 Trinity and 1,921,424 Klamath river natural origin juvenile chinook. When these population estimates are multiplied by the rotary trap mortality rates, the results are an additional 31,000 and 41,000 natural chinook mortalities in the Trinity and Klamath Rivers respectively. The total mortality estimate for both the Trinity and Klamath Rivers is approximately 240,000.

Table 2. Hatchery and natural origin juvenile chinook population mortality estimate resulting from disease in 1998.

River	Hatchery Release #	Screwtra % Mortality	Population	Hatchery Estimate	Natural Estimate	Total
Trinity	4.3 M	1.4	2.2 M	61,000	31,000	92,000
Klamath	5.1 M	2.1	1.9 M	107,000	41,000	148,000
						240,000

Although we assumed the rotary screw trap mortality rate is equal to that of the migrating population, one circumstance makes this assumption unlikely. We did not consider chinook that die upstream or downstream of the traps and are not included in the mortality estimate. This would make our mortality estimate low.

Controlled experiments which establish direct links between environmental stressors (elevated water temperatures and low oxygen) and fish disease would enhance understanding fish health in the Klamath basin. Additionally, 24 hour monitoring of dissolved oxygen and temperature at trapping sites would help our understanding of environmental stressors in the basin.

<u>In appreciation</u> This work would not have been possible without cooperation from the fish trapping crews of the Yurok Tribal Fisheries, Karuk Tribal Fisheries, U.S. Forest Service, and the California Department of Fish and Game.

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Appendix 1. Organosomatic analysis criteria scores

Skin

O = normal scale number, no lesions

1 = some scale loss, 11 - 30 % of body surface

2 = focal hemorrhages, lesions, scale loss > 30 % of body

Eye

O = no abnormalities

1 = missing 1 eye, diminutive, external abrasion, some opacity

2 = exophthalmic 'pop-eye', cataract, bubbles, parasites

3 = hemorrhage, rupture

Gill

O = normal condition, color

1 = pale

2 = clubbed, frayed, nodules, mild parasite load

Hemor. Organs

Notes about any hemorrhagic organs- abnormal size / color

N = no, Y = Yes

Appendix 2. Annual Average daily Trinity River Flow at Hoopa during October through September with corresponding ranks (1= highest, 11=lowest)

3 = necrotic zones, fungi or bacterial lesions, hemorrhagic

	Trinity River Flow at Hoopa USGS	station #11530000	
Year	Daily Mean Flow	Index	
1998	9313	1	
1997	6430	3	
1996	6202	4	
1995	7825	2	
1994	1935	10	
1993	5532	5	
1992	2035	9	
1991	1710	11	
1990	2437	8	
1989	4303	6	
1988	2798	7	

Annual Average Daily Klamath River flow at Orleans during October through September with

corresponding ranks (1= highest, 11=lowest)

Klamath	River at Orleans (USGS st	ation # 11523000)
Year	Daily Mean Flow	Rank
1998	12764	2
1997	12788	1
1996	10390	4
1995	10769	3
1994	3192	10
1993	9003	5
1992	2931	11
1991	3350	9
1990	4689	7
1989	7589	6
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Appendix 3. Trematode snail supplementary Information

In September 1996, yearling chinook from Trinity River Hatchery were exposed for 27 days to Juga sp. snails carrying an infective stage of the trematode Nanophyetus salmincola. Little mortality occurred to the exposure group and all fish became infected with the metacercaria stage of the parasite. Severity of infection ranged from 32 to 10,220 metacercaria per gram of kidney tissue with over 50% of the infected fish having > 5,000 metacercaria / g. No significant difference in blood cell numbers or composition, or plasma concentrations of protein, glucose, and triglyceride was detected between infected and control fish. Infected fish showed an increase in immunoglobulin and a decrease in saltwater osmoregulation ability. This study demonstrates that juvenile chinook can withstand severe infections of N. salmincola in freshwater, however, saltwater survival is likely to be impaired. A direct correlation between the number of metacercaria in the kidney (threshold level) and plasma sodium was not identified in the short-term saltwater challenges.

The life cycle of N. salmincola starts with the release of eggs from the adult trematode into the intestine of its final host, a piscivore such as an otter, bear, raccoon, heron, merganser, etc, and pass out into the water with feces. A ciliated miracidium stage hatches from the egg, penetrates a snail host (Oxytrema = Juga sp.), asexually multiples, and eventually produces a xiphidiocercaria (cercaria with oral sucker stylus which is motile by use of its tail). The cercaria will seek out a fish host and rapidly burrow into the skin, lose its tail, and migrate through the circulatory system to various tissues such as the gill, heart, liver, muscle, optic nerve, and kidney.

The parasite (now referred to as metacercaria) tends to concentrate in the posterior kidney, probably due to the migration path through the renal portal system (Milleman and Knapp 1970). The metacercaria will remain with the salmonid fish throughout its salt water phase and will complete its lifecycle when the fish is eaten by a final host. The longevity of the metacercarial stage has been used as a biological tag for steelhead caught in the central Pacific ocean (Dalton 1991).

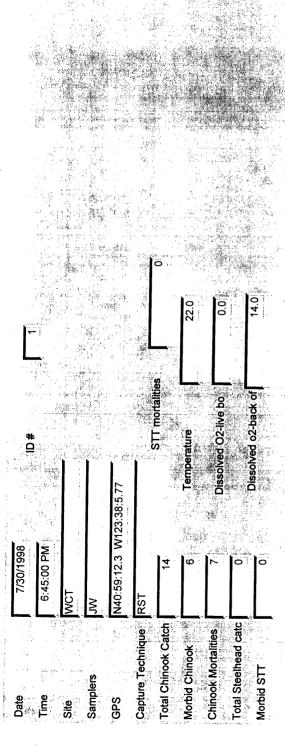
Appendix 4. Salt Water Challenge

Fourteen out of fifteen chinook survived the 80+hour saltwater challenge (see data form ID# 29 in Appendix 2). One out of fifteen fish had lesions associated with a myxosporidian infection. It was assumed that the myxosporidian would be more abundant in the kidneys of sampled chinook in 1998, as in 1997 sampled chinook. No other significant results were found.

Appendix 5. Ceratomyxa shasta

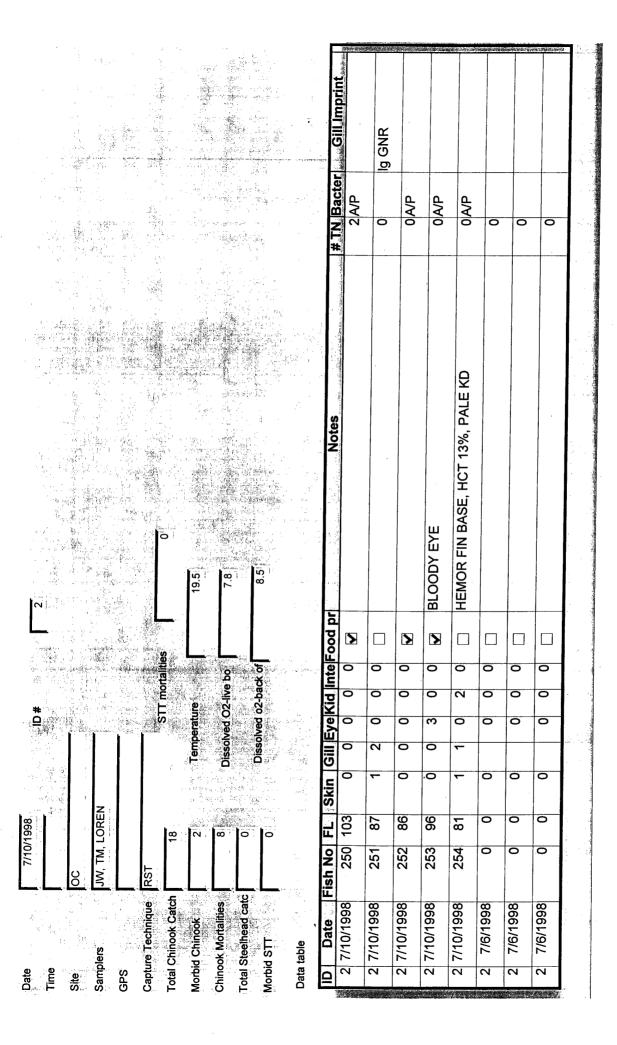
In response to the Ceratomyxosis data gathered in 1995, Mel Willis (CDFG Fish Pathologist) conducted a challenge experiment with Iron Gate Hatchery chinook in July 1996 (August 1, 1996 memo, Appendix). Chinook were held in *C. shasta* infective water source at Crystal Lake State Fish Hatchery for over 40 days (Noble 1950). The temperature of this water source does not exceed 16 ° C. There was no mortality or clinical signs observed in the chinook group. A cohort group of rainbow trout juveniles (Shasta strain which are susceptible to ceratomyxosis) held at the same site developed clinical symptoms and experienced mortalities. Histological examination of several chinook at the end of the challenge did not reveal any *C. shasta* parasites. Iron Gate Hatchery steelhead challenged at the same site for 85 days were also resistant to infection and disease. When water temperatures are under 16 - 17 °C, it appears that these Klamath R. salmonids are resistant to this endemic parasite. There are numerous reports of salmonid stocks, native to *C. shasta* infective areas, having innate resistence to ceratomyxosis (Ibarra et al. 1991).

Appendix 6. Data sheets (see pages to follow).



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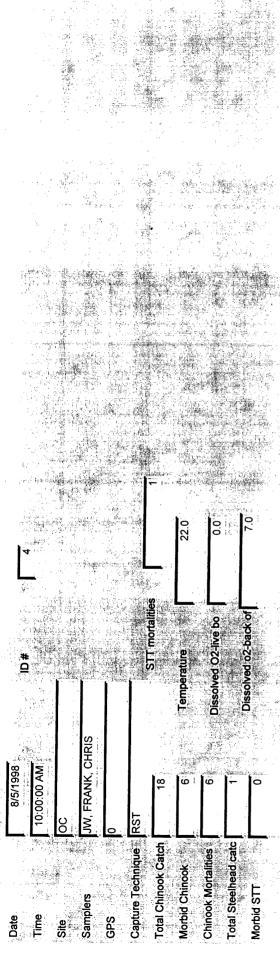
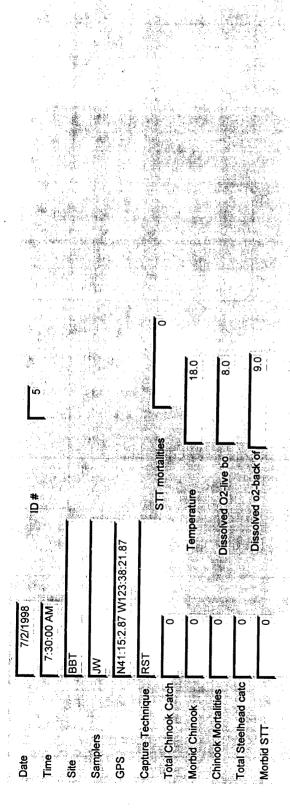


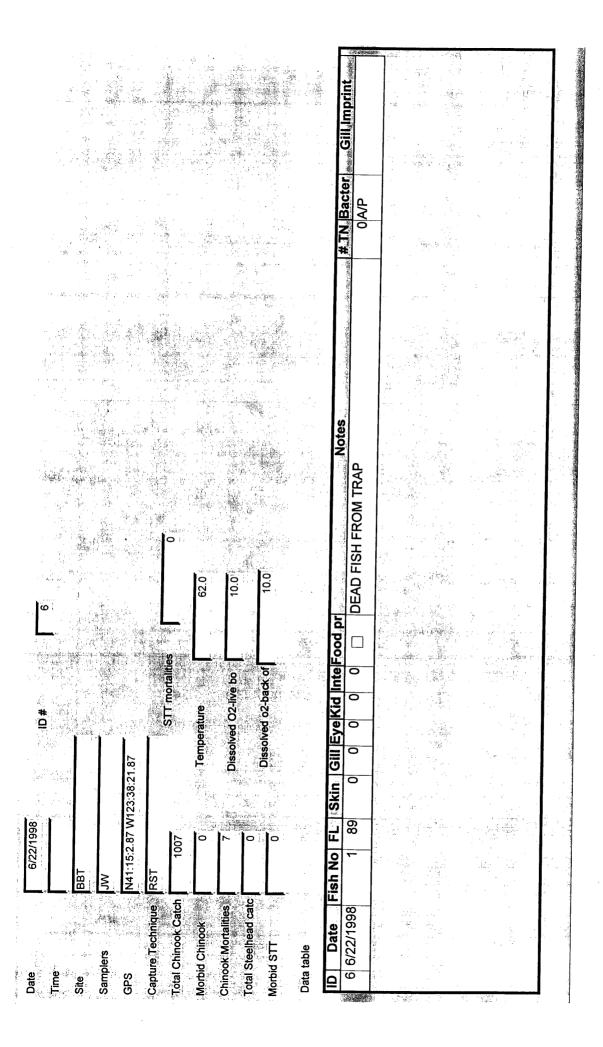
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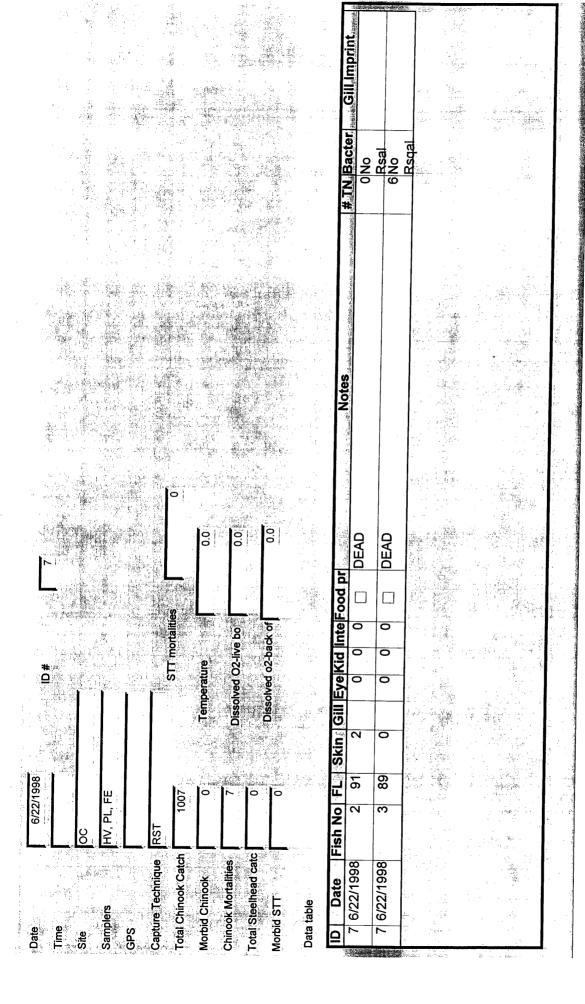
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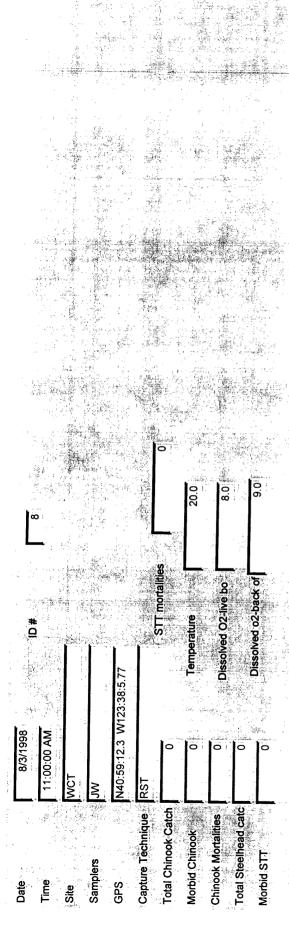


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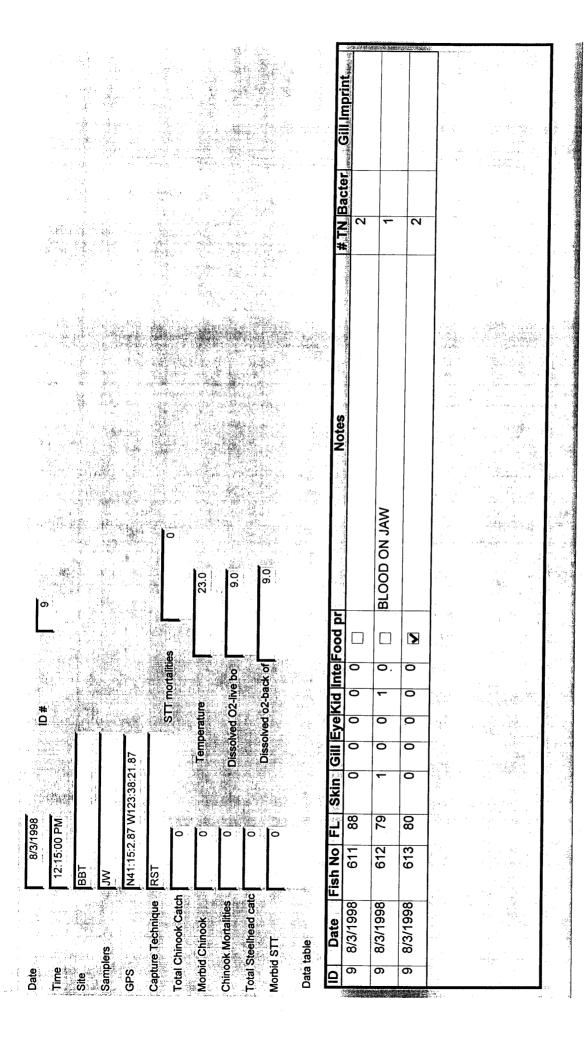
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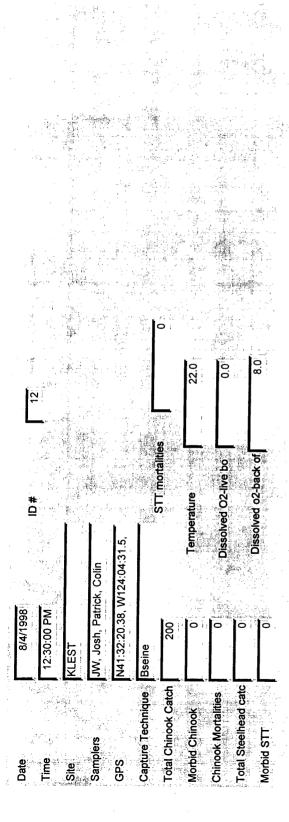
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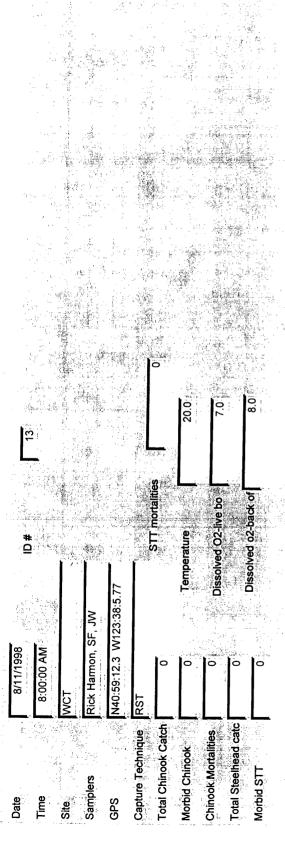
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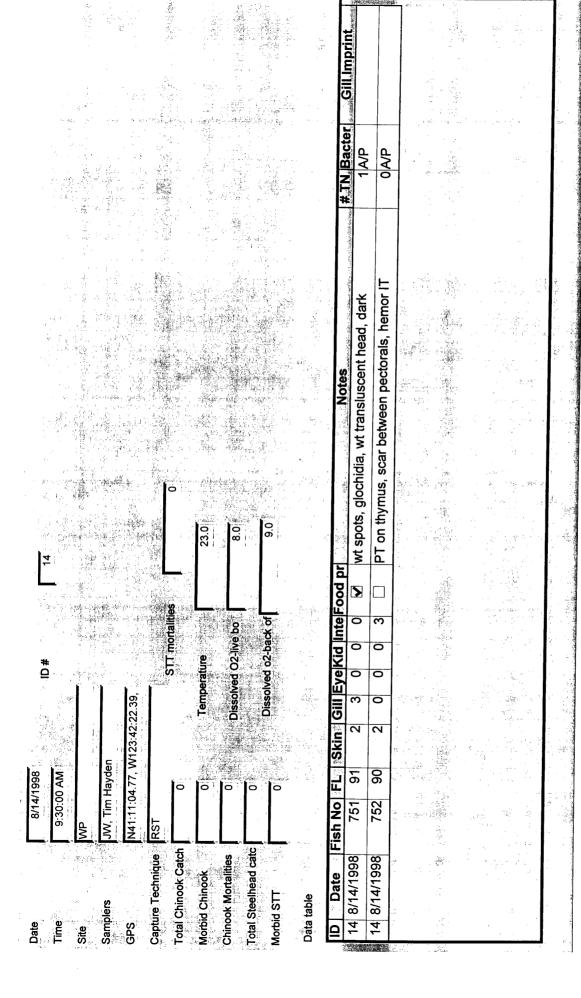
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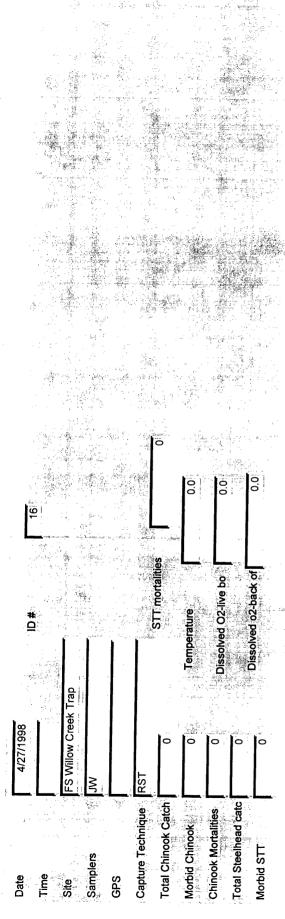
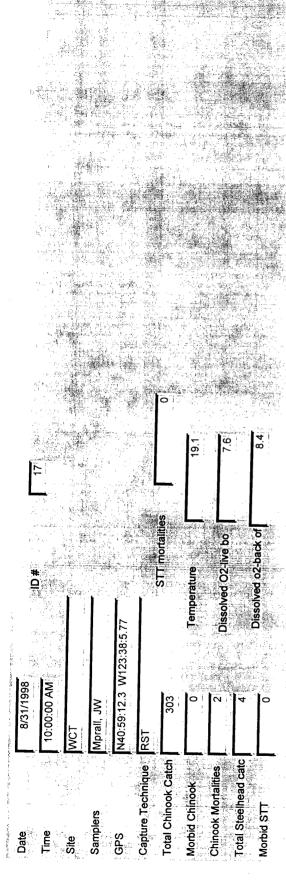


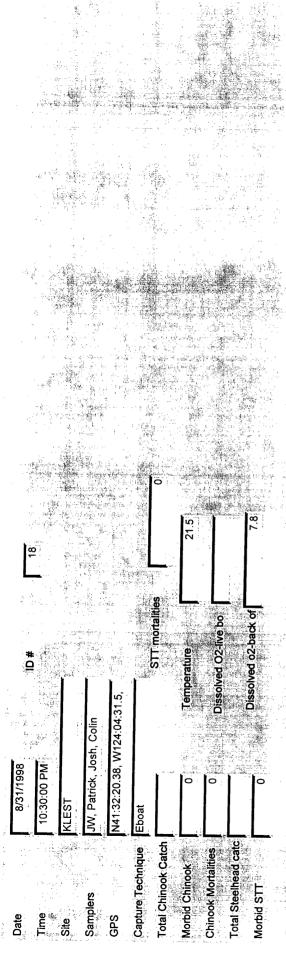
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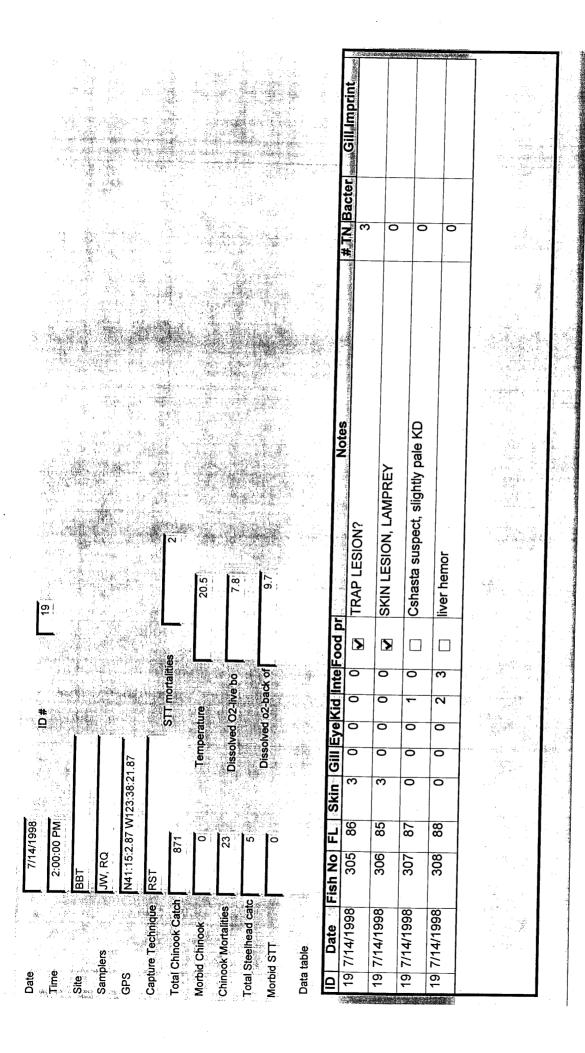
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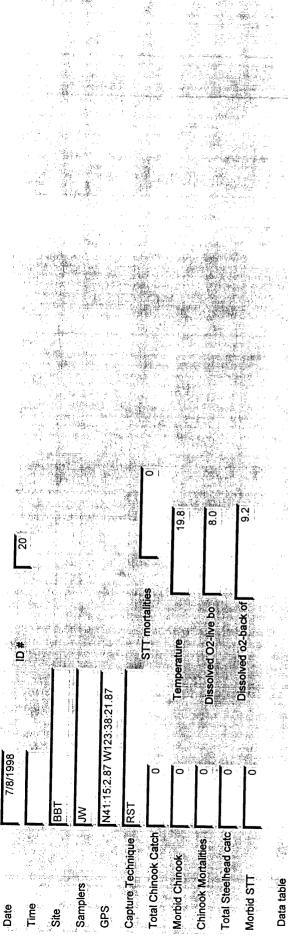
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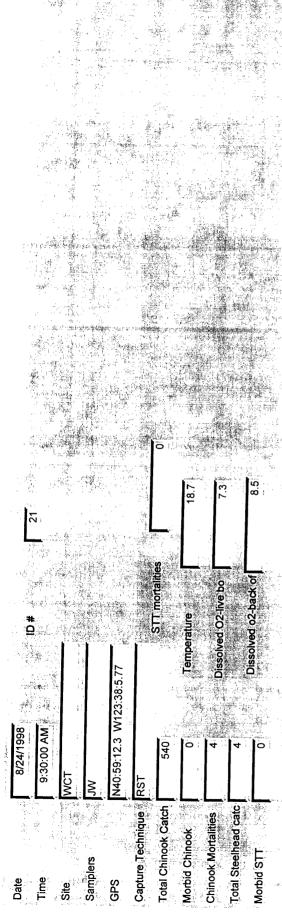
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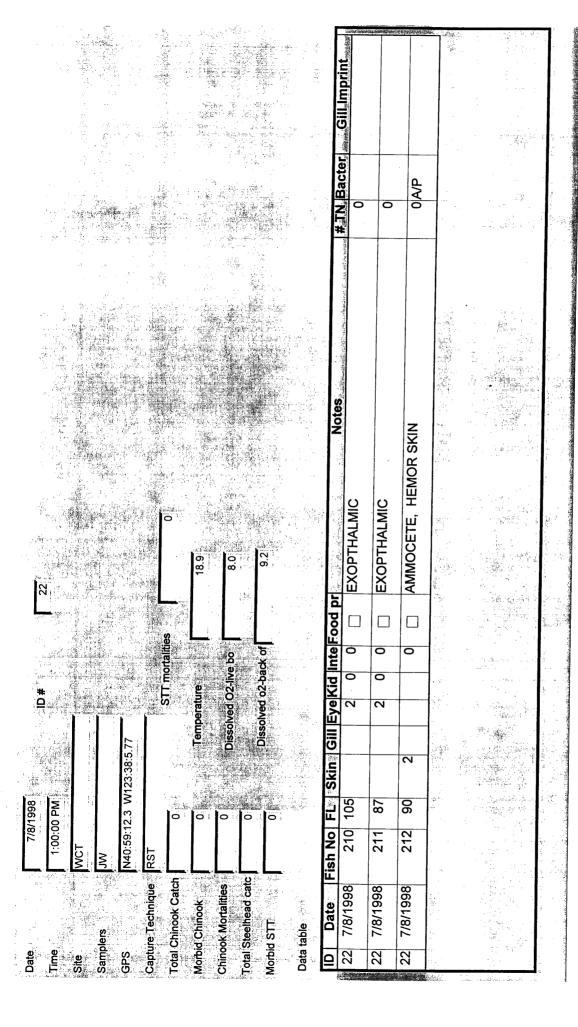
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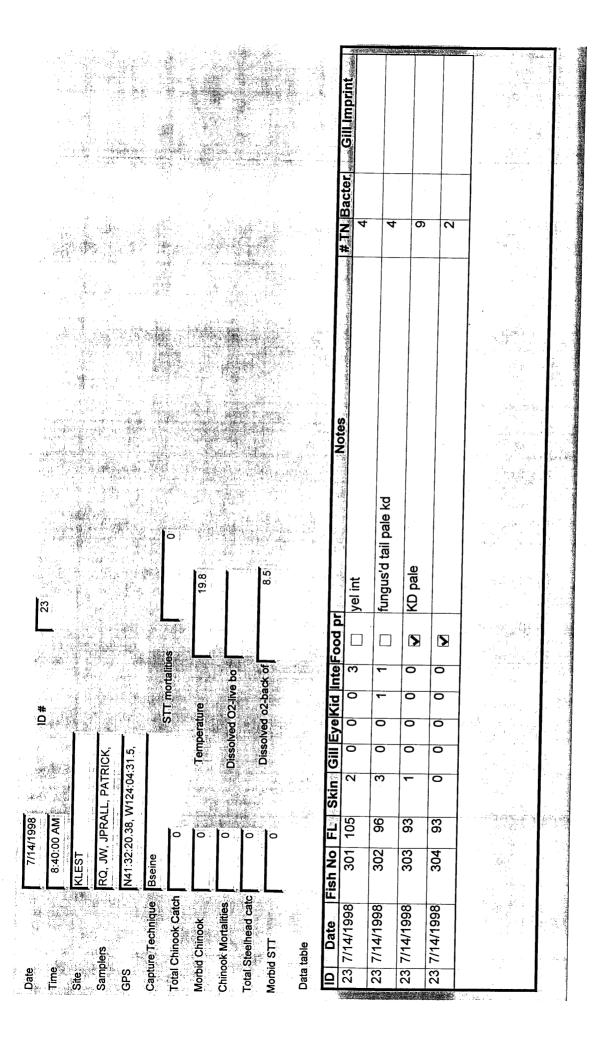
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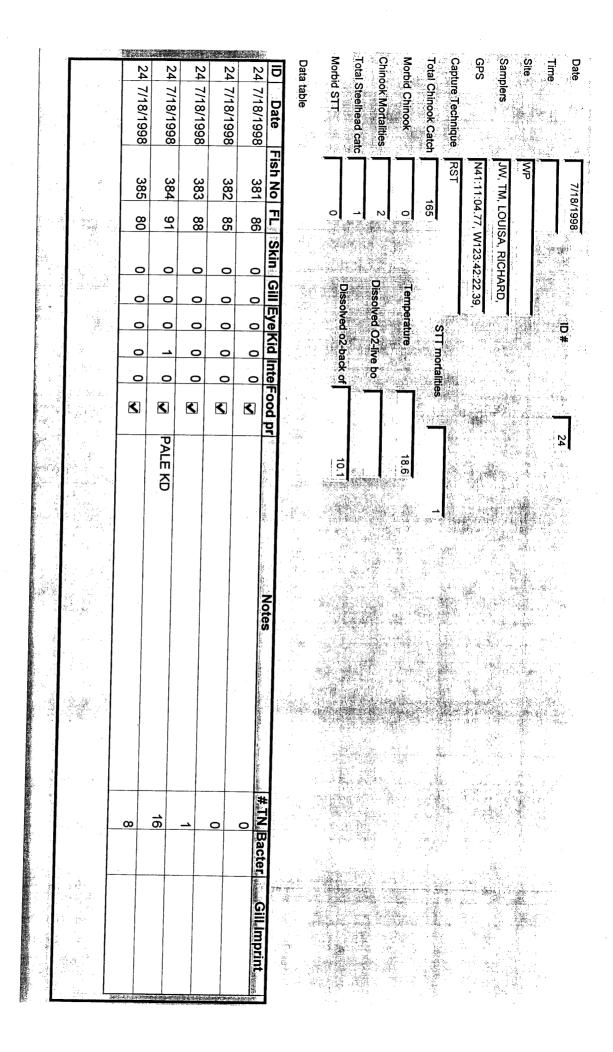


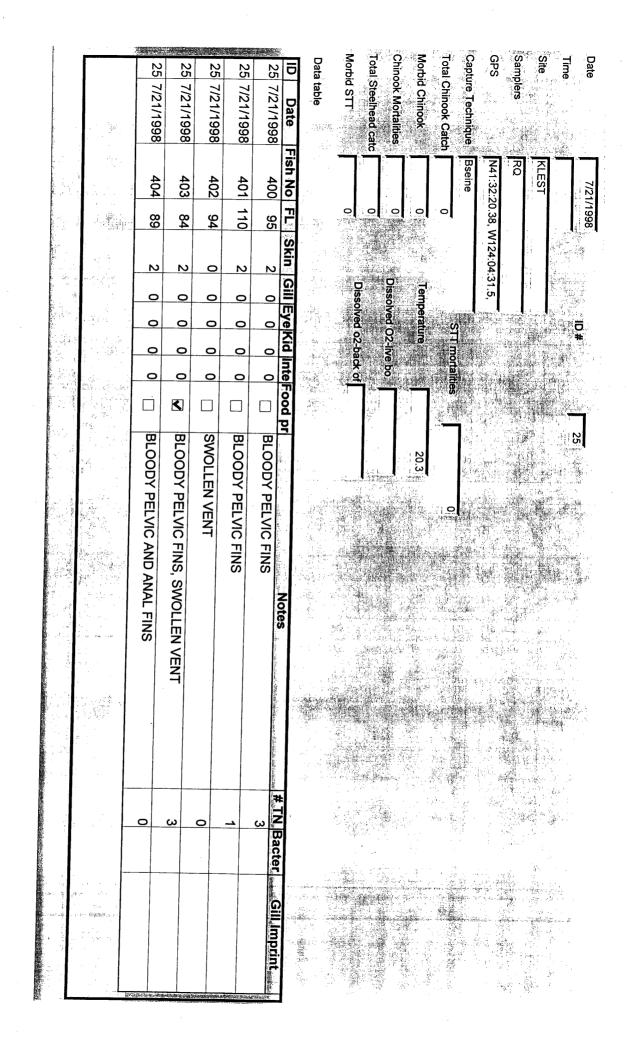
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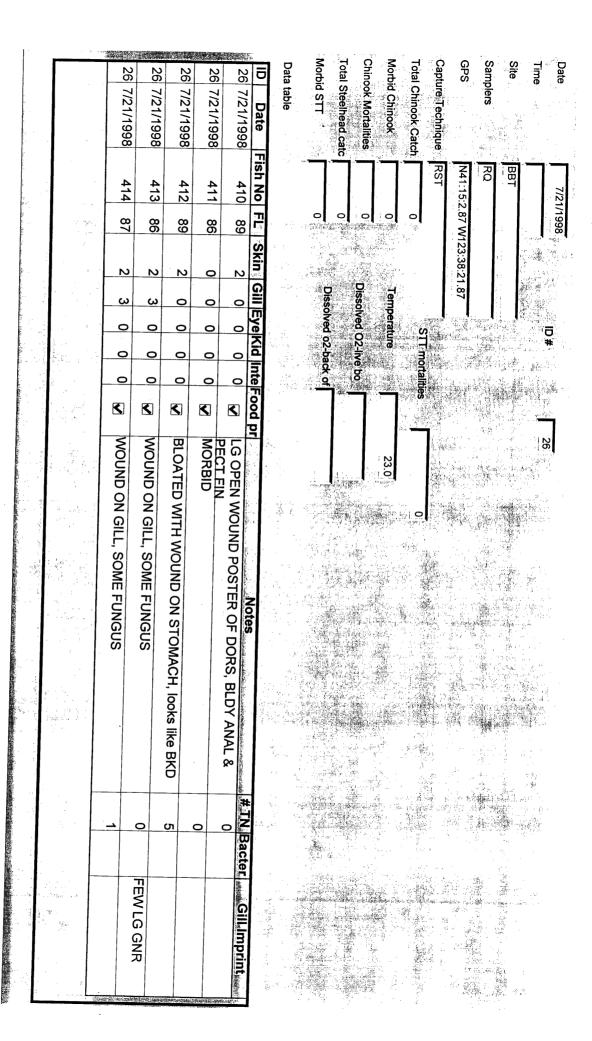
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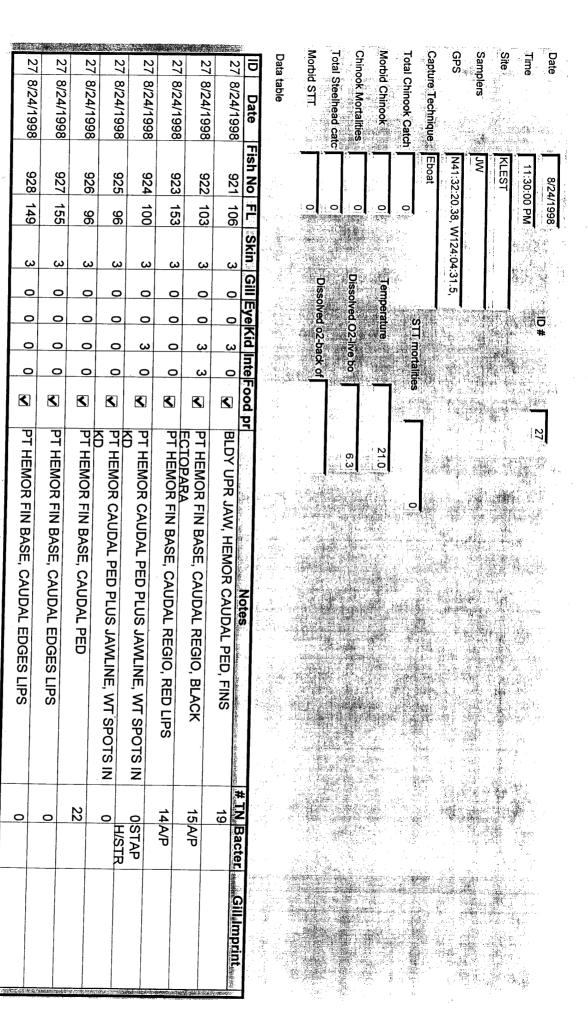


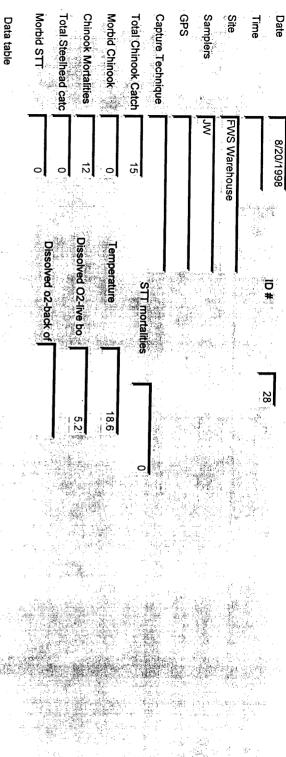




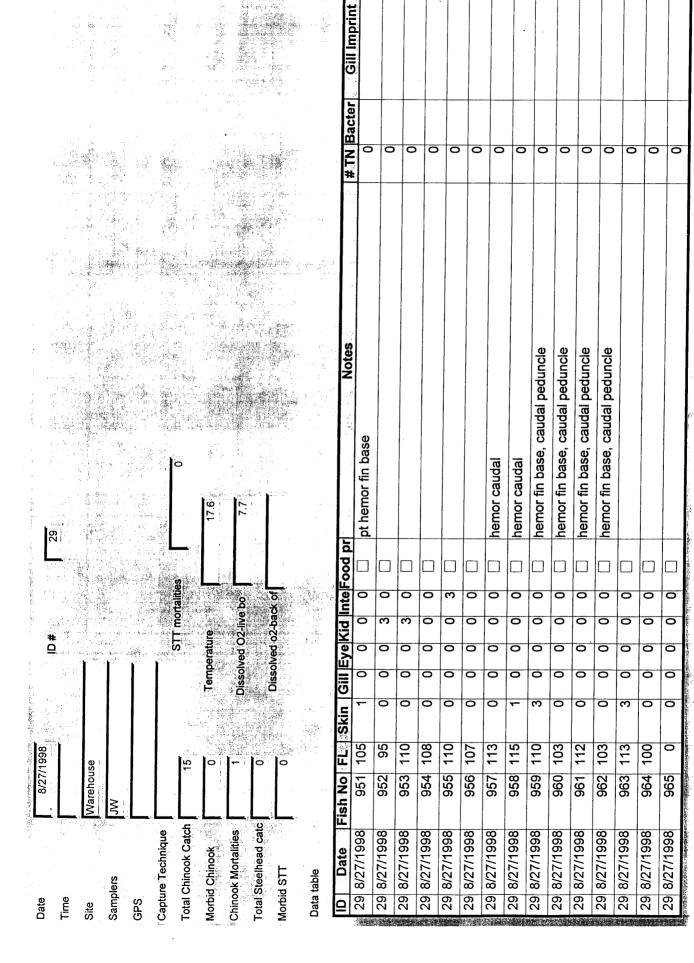








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